ADHESION OF FOREIGN PARTICLES TO PARTICULATE ANTIGENS IN THE PRESENCE OF ANTIBODY AND COMPLEMENT (SEROLOGICAL ADHESION)

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The subject of this review is the phenomenon of the adhesion of extraneous microscopic particles to particulate antigens in the presence of antibody and complement. In the traditional serological agglutination reaction specific aggregation of reacting antigen and antibody takes place without the participation by any foreign particles present by chance in the environment. There are, nevertheless, numerous published observations of the occurrence of extraneous adhesion in antigen-antibody systems. It is a purpose of this review to bring together these observations, since the literature is widely scattered and has gone generally unnoticed in the last decades. The review will consider the question of whether this type of adhesion plays a significant role in the response of the immune host to invading microorganisms. In addition a number of miscellaneous observations will be recorded which though made incidental to the study of other subjects appear to be examples of adhesion requiring the presence of both antibody and complement. Possibly the review will serve to remind investigators of an interesting serological phenomenon that it may be worthwhile to explore anew.

HISTORY OF THE ADHESION PHENOMENON

The concept of the serological adhesion phenomenon developed with the study of blood platelets. Probably the first description of antibody dependent platelet adhesion to bacteria was given by Levaditi (70) in 1901. In a study otherwise devoted to the possible origin of complement in plasma from white cells he noted *in vivo* adhesion of blood platelets to *Vibrio cholerae* in the blood stream of immunized rabbits and guinea pigs. Vibrios exposed to heat inactivated immune guinea pig serum, washed, and then injected into normal guinea pigs also became loaded with adherent platelets. These results were confirmed by Aynaud in 1911 (4) who probably was also the first person to report the *in vitro* occurrence of the Levaditi phenomenon. Nonetheless, interest in the phenomenon lagged until 1917, when Delrez and Govaertz (26) in a study of the agglutination and removal of bacteria from the blood stream, noted that bacteria in the circulation became enmeshed in clumps of platelets. The studies arising from these observations were reviewed in detail by Roskam (97) in 1927.

In 1917 Rieckenberg (94) proposed a test for trypanosomiasis employing what he considered to be a new immunity reaction. The procedure consisted of the addition of whole citrated blood to be tested for antibody to blood from an infected animal containing living trypanosomes. In a positive test adhesion of the platelets to the trypanosomes was observed microscopically. Rieckenberg was presumably unfamiliar with the French studies on the Levaditi phenomenon, and, in fact, had been anticipated in the trypanosome reaction by the French parasitologist Laveran. Laveran and Mesnil (66) in 1901 had described the agglomeration of *Trypanosoma lewisi* by blood from immune animals and had noted in the agglutinated masses entrapped leukocytes or platelets. When trypanosomes were injected into the abdominal cavity of an immune rat the exudate was observed to contain trypanosomes stuck by one of their ends to leukocytes. Similar observations with trypanosomes were reported by Mesnil and Brimont (78) in 1909.

Rieckenberg's paper was the stimulus for much activity during the next three decades. The principal studies devoted to the examination and improvement of the technique were done in Moscow, Russia, by Kritschewski and his colleagues. Their reports, accessible in a series of 24 papers, established the specificity of the antigen-antibody reaction, the requirement for complement, and the knowledge that particles other than platelets could be utilized as indicators of adhesion. They provided a reproducible technique for the test and applied it to trypanosomes, leishmanias, leptospiras, and relapsing fever spirochetes (12–17, 21, 37, 38, 55–64, 67, 79, 96, 98, 104). The adhesion

test was used in England (9, 10), Africa (95, 107, 111), Japan (77), Australia (73), and the United States (89, 101).

When reliable traditional diagnostic serological methods for trypanosomes and leptospiras were thoroughly explored, and large amounts of these organisms could be gathered for agglutination and other tests, the adhesion reaction fell into disuse and is now all but forgotten as a diagnostic test. It should be noted, however, that comprehensive attempts have not been made to compare the information obtainable from the adhesion reaction with that obtainable from other tests. The phenomenon itself is a valid one and may well deserve to be reconsidered.

The identity of the Levaditi and Rieckenberg reactions has never been clearly established. The Russian investigators (54) believed the reaction with trypanosomes was different from the Levaditi phenomenon since the latter could be demonstrated with normal animals. This argument is a weak one because the Levaditi reaction has been studied with cocci and enteric bacilli, organisms for which so-called normal animals frequently do possess antibody. Grünbaum (38) found that with paratyphoid B bacilli and normal animals the Levaditi phenomenon required the presence of heat labile antibody. He considered the naturally occurring required factor to be an antibody and not complement. With the intention of specifically removing an antibody and not complement, he used washed bacilli for absorption of normal sera in the cold and obtained adhesion to platelets with these bacteria. Grünbaum concluded that the Levaditi phenomenon, unlike the Rieckenberg reaction, did not require complement. Unfortunately this conclusion was based on the premise, rather than experimental proof, that his method of absorption of serum by bacilli in the cold removed only a naturally occurring heat-labile antibody and not any complement. Govaertz (34) and Tocantins (103), who reviewed the question, concluded the two reactions to be qualitatively identical in their natures.

Nomenclature. The term Rieckenberg reaction has generally been applied to the serological adhesion phenomenon. On the basis of priority this is inappropriate, and, particularly in France, the terms *Levaditi phenomenon* (97) or *Laveran-Mesnil reaction* (81) have been used. These usages presuppose the identity of the observations. The Russian workers have frequently used the label *thrombocytobarin reaction* invented by Kritschewski (54). This designation would seem inappropriate and possibly confusing since the specific antibody involved in the adhesion of platelets to microorganisms is directed against the microorganisms rather than the platelets. When it was shown by Brussin (11) that platelets were not the only kind of indicator particle capable of acting in the reaction, the names *Brussin reaction* and *Beladung reaction* were employed by Russian workers. The latter has been translated as *platelet loading reaction* (90). Davis and Brown (25) suggested the expression *adhesion reaction*, a properly descriptive term that does not entail any implication of historical priority.

In 1952 the term *immune adherence* was coined by Nelson (83) to describe a presumably new reaction of bacteria limited to adhesion with human erythrocytes in the presence of antibacterial antibody and complement. The term *TPIA* (*Treponema pallidum* immune adherence) has been used for the reaction in syphilis. Previously Duke and Wallace (28), and Wallace and Wormall (108) had described as *red cell adhesion* the sticking of erythrocytes to trypanosomes in the presence of specific trypanosomal antibody and complement. As in the TPIA test complement was absolutely necessary, and rarely was adhesion noted with other than primate red blood cells (man, baboon, monkey). Thus the reaction described as *immune adherence* is similar to the *red cell adhesion* of these English authors who were stimulated to undertake their studies by the observation by Leupold (69) of the occasional sticking of red cells (non-primate) to sensitized trypanosomes. The identification of immune adherence with the previously described serological adhesion phenomenon would seem to have been definitively settled by Lamanna and Hollander (65), who showed that with sensitized *Treponema pallidum*, contrary to the claims of Nelson, adhesion of inorganic and organic particles other than human red cells can take place under the proper experimental conditions and requires both antibody and complement. In view of these facts it is desirable not to employ the term immune adherence. A decision not to employ this term is also wise because it remains to be settled that in fact the adhesion of erythrocytes to sensitized microorganisms acts in a way to protect animals against infection.

Since in modern English usage adhesion as a noun is the more common term for the physical

sticking together of things, while adherence is commonly used to describe a mental or moral attachment, our adherence will be given to the name adhesion reaction.

PROCEDURE FOR OBSERVING ADHESION

It is reasonable to expect adhesion whenever a particulate antigen, a corresponding antibody, and complement meet within a living host. *In vivo* observation has rested simply on the intravenous injection of microorganisms into immunized animals followed by periodic microscopic observation of wet or stained preparations of blood samples. Most frequently platelets have been observed to be the adhering particles, blood dust and white cells not infrequently, and red cells rarely. No criteria have been offered for determining directly when *in vivo* adhesion is not due to an antigen-antibody reaction. It would be desirable to have such criteria since platelets are potentially capable of adhesion to some foreign surfaces in the absence of antibody (100).

The adhesion phenomenon has been studied principally when the reactants are isolated *in vitro*. The reaction has an advantage, when compared with some other antibody reactions, in that a relatively small number of particles of antigen are required. Investigators working with organisms difficult to obtain in large numbers may find the adhesion reaction a potentially useful serological test.

The performance of *in vitro* tests is technically simple. One merely mixes, on a slide or in a test tube, the organisms to be tested with specific antiserum, a source of complement and a suspension of indicator particles. After a period of incubation the mixture is examined by staining or directly with a microscope. It is convenient to place a droplet of the specimen to be examined on a slide and to cover it with a coverslip the edges of which are sealed with melted paraffin. This prevents evaporation and convection currents and permits observation at leisure. The slides need not be examined immediately after preparation since in our experience adhesion tends to remain unaltered at room temperature once it has taken place.

Not all authors have employed routinely the same conditions of incubation temperature and time. Temperatures have been employed in the range from room temperature (94) to 38 C (4), and the time from a few minutes (57) to overnight incubation (88). Detailed studies are not available as to how important variations in the time and temperature of incubation are in influencing the occurrence of adhesion. With *Treponema pallidum* we have found the degree of adhesion to be maximal after 120 minutes at temperatures above 25 C and below 40 C.

An indirect *in vitro* procedure has been employed by Nelson (84, 85), who, following Maaløe's (74) technique for measuring phagocytosis, centrifuges the test mixture after incubation at an empirically determined low speed so as to clear the fluid phase only of microorganisms with adherent indicator particles. The difference in counts of organisms in suspension before and after centrifugation presumably is a measure of the degree of adhesion. Obviously it is necessary to adjust the concentration of reagents critically so as to induce adhesion without concurrent significant antibody agglutination of the test organisms. Possibly, inability to meet this technical requirement may explain some of the difficulties with reproducibility experienced by some workers (23, 82, 87) with this method.

Adhesion can be observed macroscopically. With uniformly opaque suspensions of platelets adhesion to bacteria is indicated by a change to an agglutinated or flaky appearance (31, 33). Recently Daguet (24) has used the appearance of the pattern of settled human red cells at the bottom of tubes as an index of adhesion. In unpublished observations with colloidal suspensions of silica Lamanna and Hollander have found macroscopic clump formation to take place in adhesion reactions with *T. pallidum*. Unfortunately, normal guinea pig serum often contains a heat labile factor (complement ?) capable of agglutinating silica particles. Thus with silica macroscopic appearance is not a reliable index of adhesion when guinea pig serum is employed as a source of complement.

The absolute concentrations and ratio of sensitized microbes to indicator particles have been adjusted by authors to suit their convenience. A more critical attitude would seem justified since standardization of the concentrations of trypanosomes and indicator red cells has been shown to be necessary for consistent results (9). Information is lacking on how the optimum ratio of concentrations of antigen and indicator particles might be influenced by variations in antibody and complement concentrations.

THE REACTANTS FOR ADHESION

Microbes reported as showing adhesion. Protozoa, spirochaetes, eubacteria (67) and a species of filaria (88), have been reported to give the adhesion reaction with specific antisera. Unfortunately little information exists on the identity of the antigens of these organisms involved in the adhesion reaction. Kourilsky *et al.* (50) treated *Micrococcus pyogenes* var. *aureus* with papain to destroy the agglutinogen; yet these enzyme-treated cocci continued to show adhesion to human erythrocytes, and correlatively were capable of absorbing antibody concerned with adhesion, but not agglutinin. Thus it is quite possible that on occasion the adhesion reaction can be the means for revealing a surface antigenic constituent not detectable by serological agglutination.

The adhesion reaction has been negative with sera taken from infected animals when non-motile trypanosomes and leptospiras have been employed as antigen (46, 51, 62). Yet immunization with dead vaccines of trypanosomes and spirochaetes will result in production of antibody participating in adhesion (62, 77). These apparently contradictory facts may be the result of the existence of antigenic differences between living and dead microorganisms. If so they would be indicative of the power of the adhesion reaction as a tool for antigen analysis.

With cultured strains of *Treponema pallidum* (Kazan I and II, Reiter) Aristowsky and Wsorow (3) found platelet adhesion to occur in the presence of antisera from immunized rabbits and complement. That these strains possessed both common and strain specific antigens was indicated by the pattern of cross reactions in adhesion tests. While it is open to question that these cultured strains are true representatives of pathogenic *T. pallidum*, nevertheless the utility of the adhesion phenomenon for studies of the antigenic structure of treponemes is suggested by the comparable and more extensive agglutination and complement fixation studies of Eagle and Germuth (29), who also found the Reiter and a Kazan strain to be in part antigenically related and in part dissimilar.

Cultured spirochaetes grown in media containing rabbit serum have shown a spontaneous adhesion reaction which did not occur when the rabbit serum was replaced by sera from other species (3). Since normal rabbits often harbor antibody against spirochaetes, this early experience indicated the danger of carry-over of antibody by organisms grown in environments that contain antibody. Thus in employing organisms from infected animals as antigen it is probably desirable to take the organisms at a stage of infection preceding extensive antibody production.

The adhesion reaction has been successfully employed for the antigenic differentiation of species of leptospiras (60, 106) and trypanosomes (111). With antisera prepared in rabbits the saprophytic *Leptospira biflexa* could be distinguished from parasitic species, and *L. hebdomadis* from *L. icterohaemorrhagiae* and *L. icteroides*. The latter two organisms were shown to be antigenically identical (10). These results conform to those obtained by traditional serological tests.

Because of reported failures to observe the phenomenon with antibody systems against *Amoeba endolimax* (79), salmonella (54), cocci (93) and chicken erythrocytes (54), Kritschewski (54) suggested that not all microorganisms and cells might be capable of acting as particulate antigens in the adhesion reaction. Since these negative results were not based on extensive testing, and the tests were not performed with full realization of the variables affecting adhesion, it might well be that retesting would succeed in demonstrating adhesion. In fact, adhesion of the gram negative enteric bacilli (18, 33, 38), fowl (97), sheep (32), and human red cells (99) with platelets as indicators has been reported. Govaerts (34) obtained positive results with streptococci and staphylococci, and attributed the reported failures with these organisms to faulty technical procedure.

Antibody in adhesion. The fact that adhesion of platelets to microorganisms was generally observed to be limited to infected or immunized animals suggested the participation of antibody. With trypanosomes Rieckenberg (94) observed platelet adhesion only when he employed citrated plasma rather than serum. This finding, repeated by Kritschewski and Tscherikower (61), led these latter investigators to believe that the responsible factor in "immune" blood was not classical antibody but rather a principle in fibrinogen called *thrombocytobarin*. Working with a spirochaete, Krantz (51) was able to obtain adhesion with serum, a result confirmed by others

with trypanosomes; this eliminated the need for postulating the existence of a special kind of antibody not present in serum.

The antibody required for adhesion has been shown to be like classical antibody in several respects. It is non-dialyzable (111), is able to pass through a Berkefeld filter candle without loss of activity (108), and is thermostabile (10, 25, 54, 68). As is true of bacteriolysins and hemolysins, the antibody responsible for adhesion when absorbed to trypanosomes and washed free of all traces of blood is still capable of binding the complement required for the adhesion reaction (54). It remained for Raffel (89) to show that the adhesion antibody is present in the globulin fraction of serum.

As with other antigen-antibody reactions the adhesion reaction too can be observed with naturally occurring antibody which has a lesser thermostability than ordinary antibody (38).

The antibody involved in adhesion of trypanosomes to platelets and to red blood cells is not blood group agglutinin (90, 108).

Duke and Wallace (28) employed the term *adhesin* for antibody responsible for the adhesion reaction. The use of this term is convenient, but should not be taken as a challenge to the unitarian hypothesis of the nature of antibody. There has been no critical effort made to explore the capability of particular adhesins to participate in other kinds of antigen-antibody reactions. Our belief is that definitive studies of the nature of adhesin would simply reveal another facet of the potentialities of already known antibody.

Through the use of trypanosomes as the test antigen, a large number of species of animals have been shown to produce adhesin; namely, the rat, mouse, guinea pig, rabbit, dog, goat, sheep, horse, mule, monkey and man. However, an attempt to induce adhesin production by the frog has failed (98). In the wild, in areas of Africa endemic for sleeping sickness, a variety of indigenous species of primates, ungulates, canines, and rodents were found by Johnson and Lester (48) to possess adhesin. On the other hand in a nonendemic European area Cerikover and Trivis (21) obtained negative results for *T. equiperdum* adhesin with 24 species of mammals, 8 bird species, and one species each of frog and turtle.

The transfer of adhesin through the placenta and milk has been shown in relapsing fever spirochaete infections in the mouse. At the most the congenitally acquired antibody persisted for 60 days (77, 86).

Complement in adhesion. In the early *in vitro* studies of adhesion with trypanosomes and spirochaetes complement was inevitably present since the blood of infected animals was used as a source of the microorganisms. Platelets were usually employed as indicator particles without thorough prior washing. Thus arose the question of participation of complement in adhesion. While some investigators did not think that complement was required (25), the experience of these authors was limited to trypanosomes employed as antigen which at best were incompletely freed from the blood of the infected animals. The first to attack the problem systematically were Kritschewski and Tscherikower (61, 64), who concluded that a thermolabile blood factor was necessary for platelet adhesion to trypanosomes. This conclusion was soon supported by the work of others (3, 13, 51). A most convincing series of observations was made by Wallace and Wormall (108), who established that a thermolabile substance was required. It could be inactivated either by passage through a Berkefeld filter candle or by treatment with dilute solutions of ammonia. Systems rendered adhesion-negative by these traditional methods of inactivating complement could be restored in activity by adding normal serum from such animals as guinea pigs, rabbits, baboons, and man. Heat-inactivated sera were ineffective. Brown and Broom (9) went a step further by finding that removal of any one component of complement did away with adhesion. They employed carbon dioxide to separate midpiece and endpiece fractions, ammonia to inactivate the fourth component, and cobra venom to inactivate the third component. Recent work with *Treponema pallidum* has again confirmed the necessity for complement (65, 84, 85).

If antibody-complement sensitized bacteria freed of extraneous serum are heated at temperatures ordinarily inactivating complement they remain capable of adhesion with platelets (30, 68, 97). Kourilsky *et al.* (49) mixed staphylococci, staphylococcus antiserum and complement, incubated for a short time, washed and then heated the mixture for 20 minutes at 56 C before incubation with washed human erythrocytes as indicator particles. Under these circumstances adhesion was observed. Thus it may be that

complement bound to particulate antigens is no longer heat sensitive as measured by the adhesion phenomenon. Unpublished experiments by Lamanna and Hollander with complement and antibody sensitized treponemes heated in serum at 56 C for 20 minutes did not result in observable adhesion when streptococci were employed as indicator particles. That this result was due to an effect on complement rather than some other component in the system seems probable since addition of fresh normal guinea pig serum to the inactive system resulted in adhesion. These results with the treponeme system do not necessarily challenge the prior observations reported with other bacteria. It should be recalled that treponemes are among the less heat resistant bacteria and it is conceivable that the heat sensitivity of complement bound in antigen-antibody systems is influenced by the nature and heat sensitivity of the antigen.

With few exceptions (7, 10), in the work done before 1952 citrate employed as a blood anticoagulant was present in reaction mixtures at varying concentrations up to two per cent. In the light of current knowledge of the essential role played by calcium and magnesium in complement fixation (72), this would seem to be a potentially harmful practice. Some authors have remarked on the depressive action of citrate (43, 65, 88, 97) and oxalate (30) on adhesion. It is probable that the concentration of calcium ion required for blood coagulation is considerably higher than for adhesion. Thus an amount of citrate preventing coagulation might not prevent adhesion. This would explain why adhesion can still be observed in blood and plasma in the presence of anticoagulants sufficient in quantity to prevent clotting. The data of table 1 prove that the addition of chelating agents does repress adhesion and that the inhibition is reversed by the addition of calcium and magnesium. Whether calcium and magnesium could each act alone has not been determined for the adhesion reaction.

The action of complement would seem to be directly on the antigen system rather than the indicator particle. This inference is based on the knowledge that organisms exposed to antibody and complement and then washed free of unbound reagents and extraneous fluid are still capable of exhibiting adhesion upon subsequent addition of the indicator particles (54, 85).

The amount of complement required for adhesion has not been studied critically. Different authors have had widely varying experiences. Brown and Davis (10) with a leptospiral system observed adhesion with guinea pig serum diluted up to 900 times, while Lamanna and Hollander (65) with *Treponema pallidum* failed to obtain adhesion when the fresh guinea pig serum was diluted 10 times. Some early authors recommended addition of normal blood or serum to assure a supply of complement (46, 47), but most did not add complement. Complement apparently was often present in sufficient quantity by carryover in blood, plasma, or serum used as sources of pathogenic organisms, antibody, or platelets.

Brussin and Kalajev (13) felt that it was best to use the same species and even the same individual animal as a source both of complement and platelets. They obtained the poorest results with mouse complement and platelets. Nelson (84) found normal human and guinea pig sera were equally effective, though the hemolytic titer of guinea pig serum was about eight times higher than that of the human serum. These results might lead one to ask whether or not complement is the sole non-specific factor in serum participating in adhesion and what the possibility is for a role for properdin. To speculate beyond

TABLE 1

*The inhibitory effect of versene and sodium citrate on the adhesion of streptococci to Treponema pallidum and its reversal by calcium and magnesium**

Complement	Per Cent Treponemes Showing Adhesion in Presence of Various Materials				
	Versene absent	Versene (0.005M)	Versene (0.005M) Ca^{++}, Mg^{++} (0.005M)	Ca^{++}, Mg^{++} (0.005M)	Citrate 0.1M
ml					
0	2	0	0	0	0
0.1	50	18	80	66	5
0.05	52	12	54	68	
0.025	22	8	62	54	

Each of the reagents (treponemes, cocci, heat inactivated syphilitic human serum, salts, chelating agents) was added in 0.1 ml quantities with saline added to a final volume of 0.6 ml. In the calcium and magnesium mixture, calcium was three times greater in amount than the magnesium.

* Unpublished observations of C. Lamanna and D. H. Hollander.

asking these questions is idle since experimental data are lacking for critical analysis, and knowledge of the properdin system is too recent to have influenced the design of experiments in the past.

Indicator particles. Unrelated kinds of substances have been observed to function as indicator particles in adhesion. Brussin (11) and Krantz (51) were probably the first persons to show clearly that the role of platelets was not specific since they could substitute bacteria as indicator particles. Successfully employed particles have included inorganic substances (25, 64, 65), organic colloids (25, 65), yeast, bacteria (6, 13, 51, 64), and blood elements such as platelets, blood dust (25, 28, 48) leukocytes (4, 88, 89), erythrocytes (28, 37, 84), and fowl fusiform cells (36).

The very diversity of these particles suggests that the force involved in the sticking of particles to the antigen-antibody-complement complex does not arise from identities in the surface chemical structure of these indicators. We know of no experimental studies of the nature of this force. The force of sticking may well be in the nature of an interfacial tension. The following, quoted from Duke and Wallace (28), is suggestive: "A curious feature of single-celled adhesion is the way the trypanosome from time to time slips along the surface of the cell [*primate red cell*], from the tip of its posterior end to the extreme point of its flagellum always seeming on the point of breaking away but never actually losing contact." This kind of behavior would hardly seem possible if the force of adhesion were some kind of chemical binding between the microbial surface and indicator particle. The adhesion is not due to attraction between particles of opposite electrophoretic charge (8, 35).

In spite of the number of kinds of particles capable of participation in adhesion, platelets and human red cells have been favored as indicators. In good part this reflects a tendency for workers to follow precedent. While it is true that under a given set of circumstances one kind of particle seems a better indicator than others, no serious efforts have been made to establish whether or not modifications in technical procedure would shift the balance in favor of particular indicators. Nor is it known why, in the experience of different authors with test mixtures containing white cells, erythrocytes and platelets, sometimes one and sometimes another, only, shows adhesion. It may be that the relative proportions of the various indicators to sensitized microbes is one important factor. It has been shown that the relative proportion of human red cells to trypanosomes influences the degree of adhesion (9). In a comparative study of various bacteria as indicators vibrios proved best (13).

In the employment of blood particles the choice of species as source of material appears to be important. In contrast to other rodent species, mouse platelets have been found to be poor indicators for adhesion (13). Rabbit platelets have been successfully employed when guinea pig platelets have failed (3). Platelets are notoriously unstable and it would be instructive to know if modern methods of separating them from plasma with careful attention paid to adjusting the level of calcium and magnesium ions in test mixtures would improve the quality of platelets from different species for participation in adhesion. Heating platelets at 56 C does not destroy their capacity to function as indicators (97).

Nelson (83, 84) claims that only human erythrocytes act as adhesion indicators. The earlier studies of others do not support so absolute an estimate of the virtue of human red cells. While human red cells have been successfully employed when erythrocytes from other species have failed, it is important to note the existence of experiences of positive adhesion with other species' red cells. Leupold (69) noted the rare adhesion of rodent red cells; Grünbaum (37), of nucleated chicken erythrocytes; and Raffel (89), of red cells of an occasional individual rabbit, guinea pig, and rat. Most interesting are the observations in a trypanosomal system by Wallace and Wormall (108) and by Wormall (111). Adhesion was seen with erythrocytes from some infected guinea pigs, but never with cells from normal animals. Unfortunately these observations were not pursued further. Again in the trypanosomal system erythrocytes from infected but not from normal guinea pigs have acted as adhesion indicators in the presence of added normal monkey plasma (28).

Curious too has been the direct microscopic observation of adhesion with *T. pallidum* of ghosts of red cells from the sheep, rabbit, guinea pig, and chicken, all species with whose normal red cells adhesion was not observed (65). Human erythrocyte ghosts are also able to adhere (49, 84).

Highly important is the variability in human red cells as indicators in a trypanosomal system traced by Brown and Broom (9) to the individual supplying the cells. Among 26 normal persons 17 gave adhering erythrocytes; 3 gave non-adhering cells; and 9, cells with a varying capacity for adhesion. Similarly, among 52 patients with different chronic and acute diseases 23 gave positive acting erythrocytes; 13, negative cells; and 16, cells of intermediate quality. The trait of an individual seems persistent, since both in an individual yielding satisfactory cells and another giving poor cells, each gave consistently reacting erythrocytes during a two-year follow-up study. These authors could find no correlation between the ability of red cells to adhere and numerous other properties, such as blood group and red cell count of the individual source, sedimentation rate, electric charge, salt stability, precipitation by tannin, fragility, reaction at a water-oil interface, sensitivity to lysis by complement or by lecithin in the presence of cobra venom, and darkfield and vital staining appearance. Addition of serum from a person yielding red cells capable of adhesion did not confer the adhesion property on non-adhering cells and *vice versa*. These results would recommend the employment of pools of erythrocytes from different persons when cells from individual donors are not evaluated beforehand.

The capacity of human red cells to act as indicator particles has been destroyed by exposure to tannic acid and to papain (49).

Grünbaum (36) successfully employed spindle cells from fowl as indicator particles. He argued that this finding was evidence for a genetic or biological relationship in the origin of spindle cells to platelets since both kinds of cells are capable of participating in adhesion. In view of the tremendous disparity in the kinds of particles capable of acting as indicators this seems a rather far-fetched assumption.

ADHESIN IN NATURAL INFECTION

The production of adhesin accompanies infections in situations normally conducive to antibody production. Yet only in trypanosomiasis, spirochaetal infections, and syphilis have any extensive observations been undertaken. Investigations of the question of superinfection and the state of immunity in the relapse phases of trypanosomiasis and relapsing fever have been attempted by means of the adhesion reaction (15, 55, 59). A lone report exists on adhesin in filariasis (88). The adhesion reaction has been of assistance in the diagnosis of oriental sore (80), though it has been claimed to be valueless in canine leishmaniasis (Balasheva, quoted in 22). In leptospirosis adhesin occurs but the test for its detection has been said to be too capricious for routine use (47, 106). Future attention to technical improvements might result in a more favorable evaluation.

Trypanosomiasis. The adhesion reaction with platelets or red cells serving as indicators has been employed successfully for the diagnosis of human trypanosomiasis (6, 9, 28, 111) and in a field survey of animal reservoirs (48). The adhesin produced in natural infection is species specific (25, 48, 111). Strains of particular species of trypanosome have varied in their power to show adhesion so that the employment of several rather than a single strain has been recommended for survey work in the field (111). Sera against a polymorphic trypanosome of Damba Island showed a degree of cross-reaction with *T. gambiense* and *T. rhodesiense* (107), though adhesion cross-reactions between these two species have been reported as minimal or nonexistent (111). In a survey of equine trypanosomiasis of Panama the results of red cell adhesion tests correlated well with the more traditional complement fixation and precipitin tests (101).

A number of authors have investigated by the adhesion reaction the antigenic nature of strains isolated during the relapse phases of infection (1, 12, 15, 28, 89), and though not all the data are in agreement, in general differences from the parent type have been noted for the relapse strains.

Relapsing fever. Specific adhesin has been detected in relapsing fever (11, 51, 77). Matsumoto (77) reviewed the subject and recorded in detail the Japanese experience. In experimental infection adhesin appears at about the time of the crisis of the first attack and in mice persists for 3 to 8 months. Relapse strains of the infecting spirochaete are antigenically different from the parent type as determined by the adhesion reaction (14, 56, 77), though after a variable number of relapses in an individual reversion to the parent type may be observed (77).

Treatment of infected rodents with salvarsan may result in a somewhat earlier appearance of

TABLE 2

*Effect of aging of Treponema pallidum at refrigerator temperature on the adhesion reaction in a syphilitic serum system with streptococci as indicator particles**

Dilution of Syphilitic Serum	Batch of *Treponema pallidum*								
	LL			KK			II		
	Days storage in refrigerator (0–5 C)								
	0	2	7	0	2	7	0	2	4
	% treponemes showing adhesion								
0	6	72	64	8	40	72	10	72	72
5	6	16	36	2	12	10	2	16	52
25	0	14	18	0	8	8	0	8	4
125	0	2	4	0	0	0	0	4	2

* Unpublished observations of C. Lamanna and D. H. Hollander.

adhesin (77), but this does not lead to an enhancement of immunity (17). Vaccines stimulate adhesin production without concurrent development of immunity (77).

Rosenholz (96) has shown that with relapsing fever spirochaetes grown in the body louse a positive adhesion reaction with platelets is obtained with citrated blood from immunized mice. This is evidence for an antigenic similarity of the spirochaete in both the human and insect hosts.

Syphilis. The first unquestionable demonstration of the adhesion phenomenon with pathogenic *T. pallidum* was probably made in 1930 by Krantz (52, 53) with treponemes and platelets from infected rabbits. Limited to a qualitative demonstration of the adhesion phenomenon, Krantz's work has gone unnoticed. Incidental to another purpose, Turner, *et al.* (105) reported seeing tissue debris adhering to *T. pallidum* in the presence of fresh syphilitic serum.

In 1952 Nelson (83, 84) obtained adhesion between *T. pallidum* and human erythrocytes. While he failed to observe adhesion with other kinds of particles by the indirect method of observation he employed, Lamanna and Hollander (65) found by direct microscopic observation numerous types of particles capable of adhesion to the treponemes. To date serological adhesion to treponemes has been observed by these workers with platelets, red cell ghosts, bacteria, yeasts, and particles of silica, alundum, pyrex glass, styrene, and cholesterol. Not all kinds of particulate matter are equally suitable, adhesion to *T. pallidum* being poor with spores of *Bacillus subtilis* (Ford type), yeasts and India ink. Perhaps this is not surprising with yeasts, which have a notorious reputation for anticomplementary activity. No adhesion has been demonstrable with denatured chicken egg albumin, gum mastic, or *Corynebacterium xerose* as indicators.

Consistently satisfactory adhesion to *T. pallidum* has been observed with a strain of *Streptococcus pyogenes.* The presence of streptococcal antibody has not interfered with the detection of specific antibody for the treponemes since *T. pallidum* does not act readily as an indicator particle. A slight *in vitro* aging of otherwise untreated treponemes separated from testicular tissue of infected rabbits increases the capacity of these organisms for adhesion to the cocci (table 2). Thus, it is advisable to wait two days after their separation from rabbit testicular tissue and storage in the ice chest (0–5 C) before employing treponeme suspensions in adhesion studies. Prior to these observations, Hardy and Nell (41) noted a requirement for the aging of treponeme suspensions to be employed in serological agglutination tests. Preservatives should not be added to the stock antigen suspensions of treponemes since there are indications that preservatives (including merthiolate) interfere with the specificity of the adhesion reaction.

The TPIA test or indirect method of noting adhesion with human erythrocytes has been explored as a diagnostic test for syphilis (23, 87). Technical difficulties have been noted (23), and Moser (82) has suggested a modified indirect method. The direct microscopic method (65) is technically simpler, and it would appear worthwhile for someone to undertake a comparative study of the direct and indirect method.

The antibody involved in the adhesion reaction of *T. pallidum* with human red cells and *S. pyogenes* as indicators results from syphilitic infection, and can involve antibody against cardiolipin as well as noncardiolipin antibody against treponemes (23, 65). The adhesion reaction and the treponemal immobilization test tend to parallel one another (23, 65, 87).

THE BIOLOGICAL SIGNIFICANCE OF THE ADHESION PHENOMENON

Since *in vivo* adhesion of the particulates of blood to microorganisms has been observed, it is

necessary to consider what role this phenomenon may play in infection. It is also desirable to mention briefly the possible identification of the adhesion phenomenon with certain observations made without reference to the adhesion reaction.

Platelet adhesion to microorganisms. In order to assess eventually the role of the adhesion phenomenon in natural processes in which platelets adhere to surfaces (for example, thrombus formation in the presence of bacteria as in endocarditis), it will be necessary to know when such adhesion is actually the consequence of an antigen-antibody reaction involving complement. It is not known what role the serological adhesion reaction plays in thrombus formation.

Some students of platelet adhesion to eubacteria as a hematological phenomenon have not been impressed by the work in which the role of complement was established for the adhesion of platelets employed as indicator particles. As a result, though a factor in plasma and serum identified as necessary for platelet adhesion to eubacteria has been reported to be heat labile, inactivated by shaking and by adsorption to colloids, and depressed in activity by citrate (97), it has not been said to be complement. Houlihan (44), working with streptococci and dog platelets in dog serum and plasma, was deterred from labeling the adhesion factor as complement because he felt citrate should not depress a reaction involving complement. In the light of recent work on the necessity for calcium and magnesium ions in complement fixation (72) such hesitancy is no longer necessary.

In the latest work on the subject Houlihan (43, 44) and Houlihan and Copley (45) concluded that platelets *per se* were unable to adhere to bacteria, but that adhesion in the presence of whole blood, plasma, or serum probably did not require antibody though it did require the heat labile adhesion factor. In part this conclusion was based on a lack of correspondence between the bacterial agglutinin and platelet adhesion titers of sera. This criterion is not of itself decisive since it assumes *a priori* that agglutinins and adhesins are directed against the same antigens, that agglutination and adhesion reactions are of equal sensitivity, and that the individual surface antigens of microbes play the same relative role in both the agglutination and adhesion phenomena. Houlihan (44) did not feel he could arrive at a final conclusion, since in attempts at selective agglutinin adsorption of sera "the (*heat labile*) adhesive factor is inactivated in the process of adsorption". To test for the role of antibody in adhesion by agglutinin absorption of sera it is necessary to design experiments in which any complement removed by adsorption to the antibody-bacterial complex is replaced for the adhesion trials with the sera. Since this requirement was not met, the work of these authors needs repetition with an experimental design that clearly treats antibody and complement as separately manipulated independent variables.

Because adhesion to bacteria is demonstrable with defibrinated plasma or serum there is no doubt that adhesion of platelets to bacteria does not necessarily involve a fibrin coating of platelets to render them sticky. On the other hand in the presence of whole blood or plasma the sticking of platelets to bacteria could be possible by a means not involving the adhesion reaction, for example, in the case of coagulase producing staphylococci (39). There are also situations in which the adhesion reaction cannot be involved, as in the sticking of platelets to a non-antigenic surface such as glass, or in the *in vivo* adhesion of platelets to non-antigenic India ink or quartz particles (27, 100). Whether complement without antibody is involved in the latter kind of sticking can be considered still an open question. It is interesting that anticoagulants such as citrate decrease this kind of sticking of platelets (5, 112).

Hayem in 1882 (42) observed the existence of accompanying thrombocytopenia in infectious disease, an observation since repeated in numerous kinds of infections by many investigators (103). When a disease process is accompanied by the occurrence of fairly large numbers of invading organisms in the blood stream the adhesion phenomenon might be responsible for the reduction in the total number of free platelets in the circulation. This possibility does not seem to have been explored critically by any investigator. In establishing the adhesion phenomenon as a cause of thrombocytopenia it will be necessary to prove that the sticking of platelets to microorganisms and the accompanying filtration of platelet-microorganism masses from the blood stream requires the presence of both antibody and complement.

The adhesion of platelets to bacteria has been suggested to be a useful means for clearing the blood stream of invading organisms (26, 33, 97,

103). But serious doubt has been cast upon this hypothesis by Bull and McKee (18), who found various species of bacteria to disappear as rapidly from the blood stream of rabbits depleted of platelets as from normal rabbits, both in the absence and presence of antibacterial antibody.

Though no experimental evidence was offered, Leupold (69) felt that adhesion of platelets might protect trypanosomes against phagocytosis by making it difficult for white cells to come into direct contact with opsonized organisms.

It has been claimed that platelets have the power to lyse bacteria (102), and recently plakin, an enzyme-like substance specifically lysing aerobic spore-forming bacilli, has been isolated from platelets (2). The adhesion reaction, by bringing the bacilli into intimate contact with platelets, might assist the lytic properties of the platelets. However, Tocantins (103) in a review has concluded there was little evidence for the idea of platelets acting directly in the destruction of bacteria.

Phagocytosis. As long ago as 1910 Levaditi and Mutermilck (71) presented evidence that the process of phagocytosis proceeds in independent stages, with the adhesion of objects to the phagocytes being the first stage. It is possible to visualize the collision rate between microorganisms and phagocytes as controlling the rate of the first step of phagocytosis (40). The adhesion phenomenon might then be expected to assist phagocytosis by increasing the effectiveness of collisions by keeping opsonized objects and phagocytes in contact after collision. The increased stickiness of antibody-complement coated microbes could well result in a greater number of individual collisions yielding permanent adhesion of organisms to white cells. To our knowledge investigators of the effects of complement on phagocytosis have not considered this role for the adhesion phenomenon. In this connection it is pertinent to recall that Ward and Enders (109) have attributed the enhancement of phagocytosis by complement to an increase in the rate rather than an increase in the total amount of phagocytosis. More recently Maaløe (75) has reported the dependence upon complement of the phagocytosis stimulating action of immune serum, though he does not subscribe to the point of view that the enhancement due to complement is simply a matter of increased rate of phagocytosis.

The adhesion of sensitized bacteria to human red cells has been claimed to increase phagocytosis of the bacteria (84, 85). A comparison of the degree of phagocytosis obtained *in vitro* in reaction mixtures with and without erythrocytes has been offered as proof for this contention. The data presented are not extensive in quantity, nor were they collected in a critically controlled manner. Quantitative estimations of phagocytosis require careful control of bacterial density (40). Yet in the experiments reported, the final volumes of mixtures without and with concentrated suspensions of red cells were the same and thus ignored the space occupied by the red cells. To the extent that red cells occupy space the concentrations of all the other reagents are increased, an effect which while small for each reagent may be significant since the individual effects acting together may multiply rather than be simply additive. If the adhesion of bacteria to larger particles such as red cells results in greater opportunities for collision with sedimenting white cells in the encircling menstruum during the centrifugation step included in the reported experiments, then the fact of centrifugation could be responsible for the observed enhancement of phagocytosis, rather than adhesion to red cells *per se* as visualized by the author of these claims (85).

In situ immobilization of bacteria. A number of authors have commented on the apparent ability of bacteria to adhere to tissue in the presence of bacterial agglutinin (19, 20, 91, 92, 110). Thus Cannon and Pacheco (20), noting in contrast to the situation with normal animals the tendency of staphylococci to localize in the subcutaneous tissue of immunized guinea pigs, have remarked: "localization of the microorganisms by the action of agglutinating or opsonizing antibodies is suggested as of primary importance in preventing the dissemination of the infectious agent." Rich and McKee (92) especially have emphasized the function of immune serum in localizing the spread of bacteria, independent of any walling-off activity by inflammatory processes. They made observations on microscopic sections of the inoculated skin of rabbits early in the infectious process before sufficient time has elapsed for the initiation of inflammatory responses to the presence of bacteria. None of the cited authors mentioned the possibility of a role for complement in the observations they reported. That complement does play a role in *in situ* immobilization is

strongly suggested by work on "endothelial opsonin" published in 1916 by Manwaring and Coe (76). Working with perfused organ preparations, they demonstrated the necessity for a relatively large amount of a normal serum component as well as antibody for the retention in the hepatic capillaries of several different gram positive and gram negative bacterial species. While these authors did not identify the necessary normal serum component, our present knowledge of the adhesion phenomenon makes it quite likely the substance is complement.

The importance of *in situ* immobilization as a phenomenon of immunity could rest on the commonly held idea that phagocytosis is assisted by the act of concentrating invading microbes in limited areas.

Miscellaneous. In cases of thrombocytopenia otherwise unexplained, the existence of the adhesion phenomenon suggests the desirability of searching both for the presence of antibody against homologous platelets and for antibodies against blood tissue structures to which platelets might adhere. If antibody against platelets were present, adhesion of platelets to capillary walls in various organs and to white cells might occur with a resulting reduction in the number of circulating platelets. The same reduction in number of free platelets would ensue if antibody were present against some organ tissue or lining of blood vessels with which platelets come in contact during their travels in the blood stream. For example, in idiopathic thrombocytopenic purpura antibody against platelets has been sought for with variable success. It would seem logical to seek for the presence of an antibody against a tissue or organ with which platelets normally make contact during their production and circulation.

Recently there appeared in abstract form (99) a report of *in vivo* platelet and white cell adhesion to red cells in the transfusion of incompatible human blood, with accompanying thrombocytopenia and leukopenia. The explanation for these observations may well be that the introduced foreign red cells become sensitized with antibody and complement and consequently stick to platelets and white cells.

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